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ANTIGENOTOXIC PROTECTIVE EFFECT OF VITAMIN E AND OF B-CAROTENE EVALUATED IN FEMALE RECYCLERS EXPOSED TO CONTAMINANTS OF THE *CATEURA* LANDFILL IN ASUNCIÓN, PARAGUAY

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ABSTRACT

Objectives: This study aims to evaluate the antigenotoxic protective effect of Vitamin E and β-carotene in reproductive-age women working as recyclers at the Cateura landfill in Asunción, Paraguay.

Theoretical Framework: Previous studies indicate that the mix of contaminants present at the Cateura landfill can cause genotoxic damage. On the other hand, Vitamin E and β-carotene are known for their antioxidant properties and protective effects against DNA damage.

Method: The study involved a 4-month treatment with daily supplements of Vitamin E and β-carotene, along with a complementary diet. Genetic material damage levels were assessed at the beginning and end of the treatment using the comet assay and micronucleus test as biomarkers.

Results: Following the treatment, a 64% reduction in the percentage of DNA in the comet tail was observed, indicating a significant decrease in DNA damage. Additionally, significant differences in micronucleus frequencies and other cellular abnormalities were noted before and after the treatment.

Conclusions: Continuous supplementation with Vitamin E and β-carotene demonstrated a significant genoprotective effect, reducing genetic material damage in occupationally exposed female recyclers.

Research Implications: This study contributes to providing evidence on the effectiveness of antioxidants in protecting against genetic damage in highly polluted environments.

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Originality: The research supports the practical efficacy of Vitamin E and β-carotene under occupational exposure conditions, offering an innovative approach to socio-environmental management and health protection strategies.

Keywords: Antigenotoxic Effects, Comet Assay, Micronuclei, Landfill, Vitamin E, Beta Carotene.

EFEITO PROTECTOR ANTIGENOTÓXICO DA VITAMINA E E DO B-CAROTENO AVALIADO EM MULHERES RECICLADORAS EXPOSTAS A CONTAMINANTES DO ATERRO SANITÁRIO DE CATEURA EM ASSUNÇÃO, PARAGUAI

RESUMO

Objetivos: Este estudo visa avaliar o efeito protetor antigenotóxico da vitamina E e do β-caroteno em mulheres em idade reprodutiva que trabalham como recicladoras no aterro sanitário de Cateura, em Assunção, Paraguai.

Estructura Teórica: Estudos anteriores indicam que a mistura de contaminantes presentes no aterro sanitário de Cateura pode causar danos genotóxicos. Por outro lado, a vitamina E e o β-caroteno são conhecidos por suas propriedades antioxidantes e seus efeitos protetores contra danos ao ADN.

Método: O estudo envolveu um tratamento de 4 meses com suplementos diários de vitamina E e β-caroteno, associado a uma dieta complementar. Os níveis de dano ao material genético foram avaliados no início e no final do tratamento utilizando o ensaio cometa e o teste do micronúcleo como biomarcadores.

Resultados: Após o tratamento, observou-se uma redução de 64% no percentual de ADN na cauda do cometa, indicando uma diminuição significativa no dano ao ADN. Além disso, foram observadas diferenças significativas nas frequências de micronúcleos e outras anormalidades celulares antes e após o tratamento.

Conclusões: O tratamento contínuo com suplementos de vitamina E e β-caroteno demonstrou um efeito genoprotetor significativo, reduzindo os danos ao material genético das mulheres recicladoras ocupacionalmente expostas.

Implicações da investigação: Este estudo contribui a fornecer evidências sobre a eficácia dos antioxidantes na proteção contra danos genéticos em ambientes altamente poluídos.

Originalidade: A pesquisa suporta a eficácia prática de vitamina E e β-caroteno em condições de exposição ocupacional, oferecendo uma abordagem inovadora para a gestão socioambiental e estratégias de proteção à saúde.

Palavras-chave: Efeitos Antigenotóxicos, Ensaio Cometa, Micronúcleos, Aterro Sanitário, Vitamina E, Beta-Caroteno.

EFECTO PROTECTOR ANTIGENOTÓXICO DE LA VITAMINA E Y DEL B-CAROTENO EVALUADO EN MUJERES RECICLADORAS EXPUESTAS A CONTAMINANTES DEL VERTEDERO CATEURA EN ASUNCIÓN, PARAGUAY

RESUMEN

Objetivos: Este estudio busca evaluar el efecto protector antigenotóxico de la vitamina E y el β-caroteno en mujeres en edad reproductiva que trabajan como recicladoras en el vertedero Cateura en Asunción, Paraguay.

Marco teórico: Estudios previos indican que la mezcla de contaminantes presente en el vertedero Cateura puede causar daño genotóxico. Por otro lado, la vitamina E y el β-caroteno son conocidos por sus propiedades antioxidantes y efectos protectores contra el daño al ADN.

Método: El estudio consistió en un tratamiento de 4 meses con suplementos diarios de vitamina E y β-caroteno, junto con una dieta complementaria. Se evaluaron los niveles de daño al material genético al inicio y al final del tratamiento utilizando el ensayo cometa y la prueba de micronúcleos como biomarcadores.

Resultados: Tras el tratamiento, se observó una reducción del 64 % en el porcentaje de ADN en la cola del cometa, lo que indica una disminución significativa del daño al ADN. Además, se observaron diferencias significativas en

las frecuencias de micronúcleos y otras anomalías celulares antes y después del tratamiento. Conclusiones: La suplementación continua con vitamina E y β-caroteno demostró un efecto genoprotector significativo, reduciendo el daño genético en mujeres recicladoras expuestas ocupacionalmente.

Implicaciones de la investigación: Este estudio contribuye a aportar evidencia sobre la eficacia de los antioxidantes en la protección contra el daño genético en entornos altamente contaminados.

Originalidad: La investigación respalda la eficacia práctica de la vitamina E y el β-caroteno en condiciones de exposición ocupacional, ofreciendo un enfoque innovador para la gestión socioambiental y las estrategias de protección de la salud.

Palabras clave: Efectos antigenotóxicos, Ensayo Cometa, Micronúcleos, Vertedero, Vitamina E, Betacaroteno.

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1 INTRODUCTION

Cancer chemoprevention refers to the use of chemicals or dietary components to inhibit or reverse the development of cancer. Many potential chemopreventive substances inhibit cell proliferation and induce growth arrest and apoptosis by targeting multiple cellular signaling pathways (Haque et al., 2021, p. 2). Therefore, carotenoids such as β-carotene and vitamin E supplements scavenge free radicals and reactive oxygen species (ROS), which can cause oxidative damage that leads to mutations and genotoxic effects. (Combs & McClung, 2017, p. 223).

Numerous studies have reported the ability of carotenoids to promote DNA repair mechanisms in cells with DNA damage induced by exposure to different chemical and physical agents, such as ionizing radiation (Azqueta & Collins, 2012, p.4). It has been suggested that vitamin E inhibits endonuclease activation triggered by intracellular oxidative stress and improves DNA repair capacity, maintaining the genomic stability of the cell (Claycombe & Meydani, 2001, p. 37).

On the other hand, experiments conducted on animal and human cell cultures have shown protective effects of the provitamin A β-carotene at low concentrations. Beta-carotene is recognized for its significant antimutagenic activity, acting as an antioxidant and reducing the frequency of chemically induced mutations in biological models. Experimental studies have demonstrated that beta-carotene supplementation effectively decreases mutation rates, indicating its potential protective effects against DNA damage (Aidoo, Cunningham, & Leclerc, 1995). Additionally, antioxidants present either in supplements or in foods can reduce oxidative DNA damage and thereby exert a protective effect against cancer. Evidence shows that DNA

strand breaks and other forms of genetic damage can also be diminished by treatment or dietary consumption of antioxidants (Møller & Loft, 2004, pp. 79–89). Bhagavathy and Sumathi (2012, p.110) found that treating human lymphocytes with carotenoids from the green alga *Chlorococcum humicola* reduces the induction of genotoxicity by benzopyrene. Schabath and collaborators (2004) conducted a case-control study in patients with bladder cancer measuring carotenoid levels and DNA damage in lymphocytes and found an increase in DNA damage related to a low intake of carotenoids. Zhao et al. (2006) used the comet assay to measure DNA damage in 37 healthy postmenopausal women supplemented with carotenoids, demonstrating a decrease in DNA damage. Various in vitro studies on the effects of carotenoids on the DNA repair in human cells have shown a decrease in the damage caused by numerous pollutants and radiation such as UVA (Santocono et al., 2006, pp. 207-208).

Asunción, the capital city of Paraguay, with a metropolitan population of more than two million people, has the Cateura municipal landfill as its only garbage dump. In this place, about 750 tons of solid household and industrial waste are deposited daily. The garbage is sent to the landfill without being separated or classified, a job carried out by recyclers called "waste pickers". Every day, these workers sort through the garbage, separating plastics, glass, and other recyclable objects that can be sold, allowing them to provide for their families. However, many of them live around the landfill and work anywhere from 8 to 12 hours a day, chronically exposing themselves to all kinds of toxic and sub-toxic substances present in the environment, which form a mixture of pollutants classified by Christensen et al. (1994, p. 121) in four groups: organic solvents, inorganic macro components, heavy metals, and xenobiotic organic components. Mercury, selenium, cobalt, and arsenic can also be found in smaller amounts (Christensen, 2001, p. 661).

Exposure to pollutants and high levels of UV radiation can act synergistically to damage DNA and alter genomic stability in exposed populations, such as outdoor workers (Guerrero-Navarro et al., 2024). In a previous study conducted on women working as recyclers at the Cateura landfill, significantly higher levels of DNA damage were found in these women compared to a control population (Franco de Diana et al., 2018). This damage could be accumulated by chronic exposure and combined with a lack of proper education and public policies that promote prevention programs, makes this population more vulnerable to developing transmissible and non-transmissible diseases, such as cancer (Ngo, C., et al.,2021).

2 MATERIALS AND METHODS

2.1 STUDY DESIGN

A quasi-experimental study was conducted, with the exposed population serving as its control group. The genotoxic effect or DNA damage was assessed both before and after treatment with vitamin E and β-carotene supplements using the Comet Assay and the micronucleus test.

2.2 SOCIO-DEMOGRAPHIC STUDIES OF THE POPULATION, WORKING CONDITIONS, NUTRITIONAL STATUS, AND ETHICAL ISSUES

The initial sample consisted of 40 female "waste pickers" from the Cateura landfill, aged between 20 and 60 years. However, only 30 participants completed the treatment. These individuals had worked at the landfill for more than 10 years, dedicating at least 6 hours per day to the job. Participation was randomized, with all eligible individuals given the opportunity to participate. Exclusions were limited to those undergoing oncological treatment or occasional workers.

All participants were informed about the objectives of the study, procedures, and their expected involvement through multiple community-oriented informative sessions. Participation was entirely voluntary, and each participant expressed their willingness to take part in the study by signing an informed consent form. The research ethics committee of the Faculty of Health Sciences at the Catholic University "*Nuestra Señora de la Asunción*" approved the design of the study.

A survey consisting of both closed and open-ended questions was administered to collect data on the sociodemographic characteristics of the population and potential confounding factors. These factors encompass smoking habits, alcohol consumption, working hours, and seniority.

Each research participant underwent a nutritional assessment that included measurements of weight, body mass, and height, along with a survey to assess their eating habits. This nutritional evaluation was conducted both before and after the vitamin supplement treatment.

2.3 TREATMENT WITH VITAMIN E AND B-CAROTENE AS ANTIGENOTOXIC AGENTS

Based on a previous study (Remely et al., 2017), a group of recyclers was provided with capsules of 400 I.U. of vitamin E, and tablets containing 20 mg of β-carotene. The amounts were calculated to ensure that participants received a daily dose of both vitamins for four months.

As a complement to the treatment, participants were advised to follow a diet rich in sources of the aforementioned vitamins. Information leaflets were distributed and talks on healthy eating were conducted. To ensure treatment adherence, participants were monitored throughout the 4-month period.

2.4 BIOMARKERS OF GENOTOXICITY

2.4.1 Micronucleus analysis in exfoliated cells of buccal mucosa

Cell samples from the mucosa of recyclers were collected in situ at the landfill. Before collection, each participant rinsed their mouth with water, followed by a gentle scraping of the inner cheek using a wooden spatula. The collected samples were then spread onto clean glass slides.

For each individual, three replicate samples were obtained, air-dried at room temperature, and subsequently fixed in 80% methanol for 1 hour. Staining of the cells was performed using the Feulgen technique. This involved immersion of the slides in 1N HCl at 37°C for 1 minute, followed by 10 minutes in 1N HCl at 60°C, and a final 10-minute immersion in 1N HCl. The slides were then immersed in Schiff reagent for 1 hour in the dark and rinsed with deionized water. Special microscope filters were utilized to enhance cytoplasmic contrast.

2.4.1.1 Cell evaluation criteria

Following the classification criteria of the BMCyt assay, micronuclei and other nuclear abnormalities were identified, including karyolysis, karyorrhexis, pyknosis, nuclear buds, condensed chromatin, and binucleated cells (Thomas et al., 2009; Bolognesi et al., 2017). The frequency of each of these abnormalities was determined by the total count of 2,000 cells.

2.4.2 Comet assay as a biomarker of effect for each of the participants

Peripheral blood samples of 40 µl were obtained from the ring finger of each participant. The Comet technique was performed following the protocol standardized by Singh (2016). These samples were placed onto glass slides gelled with normal melting point agarose (45°C), covered with coverslips, and allowed to gel at 4°C for one hour. Viable cells suspended in 15 µl of PBS were added to 80 µl of 0.5% low melting point agarose (37°C). After removing the coverslips, a second layer of 80 µl of the sample mixture was applied to the pre-treated slides and allowed to solidify at 4°C for 10 minutes.

Following this step, the coverslips were carefully separated from the slides, and a third layer of 75 µl of low melting point agarose was pipetted onto the slides and allowed to gel at 4°C for an additional 10 minutes. To induce membrane lysis, the slides (without coverslips) were immersed in a freshly prepared lysis solution containing 1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris-HCl (pH 10), 1% Triton X-100, and 10% dimethyl sulfoxide at 4°C for 1 hour. The slides were then positioned in a horizontal electrophoresis vessel and covered with a freshly prepared buffer solution (1 mM Na2EDTA, 300 mM NaOH, pH 12.5) for 20 minutes to allow DNA relaxation and the manifestation of alkali-labile sites. Electrophoresis was carried out for 20 minutes at 0.75 V/cm (25 V, 300 mA). After electrophoresis, the slides were rinsed in a 0.4 M Trizma base neutralization buffer at pH 7.5 to remove excess alkali.

For staining, 80 µl of ethidium bromide (10 µg/ml) was applied to each sample, and after 20 minutes, excess dye was washed away with deionized water. The slides were prepared in triplicate for each individual in the study, and 200 nuclei per participant were examined. The percentage of DNA in the tail was recorded following the latest established guidelines (16-18) and used to determine cell damage, using a Zeiss Axio Lab A1 epifluorescence microscope equipped with a 515-560 nm excitation filter and a 590 nm emission filter.

A 20x objective lens was utilized to project the image onto a highly sensitive camera. Imaging was conducted with Comet Imager v2.2 software, developed by MetaSystems in Germany. This software not only captures images but also calculates and integrates the intensity profile for each cell.

2.5 STATISTICAL ANALYSIS

Statistical analysis was conducted using IBM SPSS Statistics (version 23). To assess differences between the distributions of the two populations (before and after treatment), the Wilcoxon Signed-Rank Test, Sign Test, and McNemar Test were applied. Additionally, analysis of variance (ANOVA), F-statistics, and Student's t-test were performed to compare both treatments.

3 RESULTS

3.1 DEMOGRAPHIC CHARACTERISTICS AND NUTRITIONAL STATUS OF THE POPULATION

The participants had an average age of 42 years and an average work experience of 18 years. Among the 30 women in the study, only 6 had worked fewer than 8 hours per day. Initially, the majority were overweight, with an average weight of 68 kg and a BMI of 59. Following treatment with vitamin E and β-carotene supplements, the average weight increased by 1.72 kg.

3.2 GENOTOXICITY BIOMARKERS

3.2.1 Measurement of DNA damage by comet assay

Figure 1 shows the contrast in the percentage of DNA in the tail, an indicator of DNA fragmentation related to the genotoxic effects of environmental pollutants, in the female recycler population before and after treatment with vitamin E and β-carotene supplements. The data reveals a 64% decrease in DNA within the tail after treatment with the vitamins and the proposed nutritional plan for each participant, indicating a significant difference ($p<0.05$) (Table 1).

Figure 1

Compared distribution of DNA% in the comet tail before and after treatment

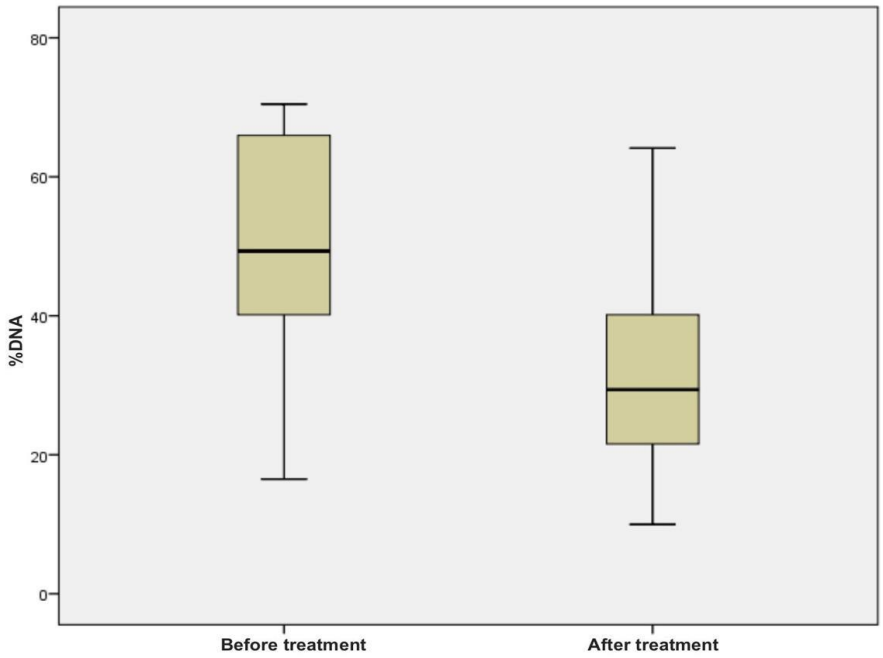


Table 1

Analysis of variance (ANOVA) of DNA% in the comet tail before and after treatment

Groups	*Count*	*Sum*	*Mean*	*Variance*
% DNA before	30	1502,95898	50,0986325	212,707692
% DNA after	30	965,705574	32,1901858	183,976053

3.2.2 Evaluation of genotoxicity by Micronucleus test in buccal mucosa cells or BMCyt

As shown in Table 2 (means and SD of MN), significant differences were observed in the frequencies of MN and other cell abnormalities before and after treatment with Vitamin E and β-carotene, as indicated by ANOVA tests ($p < 0.05$), Levene's test for equal variances, and t-tests for equality of means.

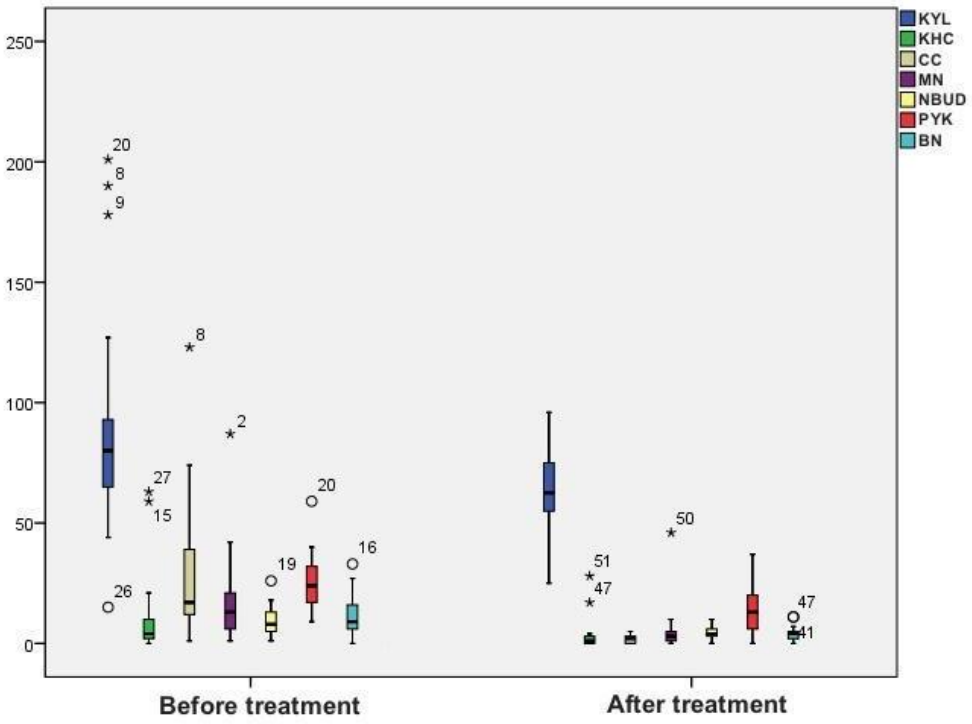
Table 2

Genotoxic assessment as measured by the Cytome assay micronucleus test in buccal mucosa cells

	Nuclear anomalies						
Treatment	KL	KH	CC	MN	BE	PK	BN
Before treatment	88,97±39.94	9,57±15,27	27,80±28,14	16,73±16,23	9,13±5,55	24,50±10,62	11,17±7,97
After treatment	63,33±16,20	2,87±5,68	1,60±1,67	4,63±8,24	4,57±2,82	14,80±9,36	3,87±2,90
Nuclear anomalies: karyolysis (KL); karyorrhexis (KH); condensed chromatin (CC); micronuclei (MN); broken eggs (BE); pyknosis (PK); binucleate (BN)							

Figure 2

Distribution of the BMCyt assay results before and after treatment. KYL: karyolysis; KHC: karyorrhexis; CC: condensed chromatin; MN: micronucleus; NBUD: nuclear buds; PYK: pyknosis; BN: binucleated cells.



4 DISCUSSION

It is evident that agents in the environment of the workplace of these garbage recyclers at the municipal dump of Cateura interact with their DNA, resulting in genotoxic activity. Studies have revealed that the percentage of DNA in the comet tail, as evaluated in peripheral

blood samples of women exposed to pollutants from the Cateura municipal dump, is higher than that observed in non-exposed women. Furthermore, the level of genetic material damage correlates with the duration of exposure. Additionally, this research has demonstrated an increase in the frequencies of micronuclei and other nuclear abnormalities, such as karyolysis and karyorrhexis (Franco de Diana et al., 2018).

The combination of organic and inorganic chemical compounds to which these individuals are exposed not only damages the DNA molecule but could also alter the DNA repair mechanisms. The Comet technique reveals both double and single-strand DNA breaks. When assessing the baseline DNA damage in working women, we observed a high level of damage to the DNA molecule, consistent with previous research indicating that contaminants induce breaks in the DNA molecule (Franco de Diana et al., 2018).

In this study, we have found that the percentage of DNA in the comet tail decreased with the treatment based on a diet rich in vitamin E and β-carotene supplements. The results obtained from the Micronucleus test align with those from the Comet test, both demonstrating a reduction in the frequencies of micronuclei and other nuclear abnormalities. This supports the positive impact of the treatment. These findings coincide with results from other investigations, explaining the decrease in the percentage of DNA in the comet tail. For example, it has been demonstrated that this treatment significantly reduces free radicals that induce chromosomal damage (Duthie et al., 1996, p. 1291). Other in vivo and in vitro studies have shown that vitamin E reduces DNA damage and enhances the reparative capacity of genetic material (Møller & Loft, 2004; Factor et al., 2000; Odin, 1997).

Factor et al. (2000) reported that vitamin E supplementation effectively reduces the percentage of chromosomal aberrations and reduces liver tumor formation in mice. Vitamin E has also been recognized for its ability to induce apoptosis in various cell lines with DNA damage (Azqueta & Collins, 2012, p. 12).

The various contaminants to which the women recyclers at the Cateura landfill are exposed may act synergistically during prolonged periods of chronic exposure. This interaction can induce chromosome breaks, leading to the formation of acentric and dicentric fragments, and subsequently, the appearance of MN, which are cytoplasmic bodies of nuclear origin that are incorrectly incorporated into daughter cells following cell division. The identification of figures such as NBUD is associated with processes of chromosomal instability and gene amplification. The higher frequency of binucleated cells observed in the exposed population may be linked to cytokinesis mechanism failures, possibly due to defects in contractile ring formation or cell cycle arrest, leading to abnormal chromosome segregation (Bolognesi et al.,

2013; 2016). Sayed et al. (2012) demonstrated that administering selenium and a vitamin supplement containing vitamins A, C, and E reduced genotoxic effects, including sister chromatid exchanges and chromosomal aberrations induced by sunlight exposure in rat bone marrow cells.

Van Helden et al. (2009) demonstrated an enhanced base excision repair capacity in ferret lung cell cultures supplemented with carotene using specific assays.

Recent research has prevented DNA damage in peripheral blood mononuclear cells of patients who consumed antioxidants before undergoing bone imaging with technetium-99m methylene diphosphate. This suggests that antioxidants may serve as a protective measure for individuals, including healthcare professionals, exposed to radiation during imaging studies (Velauthapillai et al., 2017).

Other studies on the effect of antioxidants on repair enzymes revealed an increased ability to remove oxidized purines (Møller & Loft, 2004).

Remmely et al. (2017) suggested that antioxidant intervention, combined with the epigenetic activity of antioxidant ingredients in the diet, could be an effective approach to preventing oxidative stress.

β-carotene and Vitamin E may be modulating the metabolic composition of the gut microbiota and improving the intestinal barrier, maintaining a normal immune function (Li et al., 2023). This reduces the inflammatory processes caused by oxidative stress while also reducing genomic instability.

5 CONCLUSIONS

Cells are constantly exposed to endogenous metabolic oxidants and external factors, underscoring the significance of DNA repair systems and dietary antioxidants in reducing DNA damage levels and cancer risk.

Studies assessing dietary supplementation with antioxidants and diets rich in carotenoids and polyphenolic-rich vegetables and fruits have primarily employed biomarkers such as DNA damage in white blood cells, urinary excretion of oxidized bases, and DNA repair capacity.

It is worth noting that some studies have observed oxidative effects of β-carotene at high doses, leading to increased damage to the genetic material. Therefore, caution is advised when considering antioxidant supplements, especially concerning the recommended dosage.

This research demonstrates that continuous treatment with vitamin E and β-carotene supplements reduces DNA damage, providing protective effects against genotoxic mechanisms of environmental factors that affect the recyclers at the Cateura landfill. These protective effects likely extend to endogenous factors that trigger the production of free radicals through metabolic mechanisms in response to environmental pollutants.

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AUTHORS' CONTRIBUTIONS

Deidamia Franco conceived the original idea and supervised the project. She was in charge of the overall direction and planning.

Deidamia Franco, Diana Castiglioni, Jaime Segovia, and Rafael Figueredo conceived and planned the experiments.

Jaime Segovia, Diana Castiglioni, Natalia Urdapilleta, and Miriam Schupp carried out the experiments, collected the data, and processed the samples.

Rafael Figueredo and Carmiña Cilia performed the nutritional measurements throughout the project.

Deidamia Franco, Diana Castiglioni, Jaime Segovia, Carmiña Cilia, and Juan Duré contributed to the interpretation of the results.

All authors discussed the results and contributed to the final manuscript.

Deidamia Franco, Diana Castiglioni, Jaime Segovia, Carmiña Cilia and Adolfo Monges wrote the paper.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Research Ethics Committee of the Faculty of Health Sciences at the Catholic University "Nuestra Señora de la Asunción" in Asunción, Paraguay. All participants consented to take part in this study by signing an informed consent form.

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